



SEQUENCE LISTING

<110> YEASTERN BIOTECH CO., LTD

<120> A FUNGAL IMMUNOMODULATORY PROTEIN (FIP) PREPARED BY MICROORGANISMS AND USES THEREOF

<130> PCT/CN2004/001044

<140> US 10/572,563

<141> 2006-03-17

<150> PCT/CN2004/001044

<151> 2004-09-14

<150> US 60/503,547

<151> 2003-09-17

<160> 15

<170> PatentIn version 3.3

<210> 1

<211> 336

<212> DNA

<213> Ganoderma lucidium

<220>

<221> variation

<222> (1)..(336)

<223> improved DNA sequence of G. lucidium FIP for expressing in yeast, FIP-yeast

<400> 1

atgtctgata ctgctttgat tttcagattg gcttgggatg ttaagaagtt gtcttttcgat	60
tacactccaa actggggtag aggtaaccca aacaacttca ttgatactgt tactttccca	120
aagggttttga ctgataaggc ttacacttac agagttgctg tttctggtag aaacttgggt	180
gttaagccat cttacgctgt tgaatctgat ggttctcaaa aggttaactt cttggaatac	240
aactctgggtt acgggtattgc tgatactaac actattcaag ttttcgttgt tgatccagat	300
actaacaacg atttcattat tgctcaatgg aactga	336

<210> 2

<211> 336

<212> DNA

<213> Ganoderma lucidium

<220>

<221> gene

<222> (1)..(336)

<223> original FIP codon, FIP-lz

<400> 2

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atgtccgaca ctgccttgat cttcaggctc gcctgggacg tgaagaagct ctcgttcgac    60
tacacccccga actgggggccc cggcaacccc aacaacttca tcgacactgt caccttcccc    120
aaagtcttga ccgacaaggc gtacacgtac cgcgtcgccg tctccggacg gaacctcggc    180
gtgaaaccct cgtacgcggg cgagagcgac ggctcgcaga aggtcaactt cctcgagtac    240
aactccgggt atggcatagc ggacacgaac acgatccagg tggttcgttgt cgacccccgac    300
accaacaacg acttcatcat cgcccagtgga aactag                                336
```

<210> 3

<211> 40

<212> DNA

<213> Artificial

<220>

<223> primer

<220>

<221> primer

<222> (1)..(40)

<223> forward primer of FIP-yeast

<400> 3

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aaaaaaaaaa ggatcccgca atgtctgata ctgctttgat                                40
```

<210> 4

<211> 41

<212> DNA

<213> Artificial

<220>

<223> primer

<220>

<221> primer

<222> (1)..(41)

<223> reverse primer of FIP-yeast

<400> 4

```
aaaaaaaaaa acacgtgtca actagttagt tccattgagc a                                41
```

<210> 5

<211> 41

<212> DNA

<213> Artificial

<220>
<223> primer

<220>
<221> primer
<222> (1)..(41)
<223> forward primer of FIP-1z

<400> 5
aaaaaaaaaa ggatcccgca atgtccgaca ctgccttgat c

41

<210> 6
<211> 42
<212> DNA
<213> Artificial

<220>
<223> primer

<220>
<221> primer
<222> (1)..(42)
<223> reverse primer of FIP-1z

<400> 6
aaaaaaaaaa acacgtgtca actagttagt tccctagttc ca

42

<210> 7
<211> 111
<212> PRT
<213> Ganoderma lucidium

<220>
<221> PEPTIDE
<222> (1)..(111)
<223> FIP amino acid sequence

<400> 7

Met Ser Asp Thr Ala Leu Ile Phe Arg Leu Ala Trp Asp Val Lys Lys
1 5 10 15

Leu Ser Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Asn Pro Asn Asn
20 25 30

Phe Ile Asp Thr Val Thr Phe Pro Lys Val Leu Thr Asp Lys Ala Tyr
35 40 45

Thr Tyr Arg Val Ala Val Ser Gly Arg Asn Leu Gly Val Lys Pro Ser

50

55

60

Tyr Ala Val Glu Ser Asp Gly Ser Gln Lys Val Asn Phe Leu Glu Tyr
65 70 75 80

Asn Ser Gly Tyr Gly Ile Ala Asp Thr Asn Thr Ile Gln Val Phe Val
85 90 95

Val Asp Pro Asp Thr Asn Asn Asp Phe Ile Ile Ala Gln Trp Asn
100 105 110

<210> 8

<211> 111

<212> PRT

<213> Ganoderma tsugae

<220>

<221> PEPTIDE

<222> (1)..(111)

<223> FIP amino acid sequence

<400> 8

Met Ser Asp Thr Ala Leu Ile Phe Arg Leu Ala Trp Asp Val Lys Lys
1 5 10 15

Leu Ser Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Asn Pro Asn Asn
20 25 30

Phe Ile Asp Thr Val Thr Phe Pro Lys Val Leu Thr Asp Lys Ala Tyr
35 40 45

Thr Tyr Arg Val Ala Val Ser Gly Arg Asn Leu Gly Val Lys Pro Ser
50 55 60

Tyr Ala Val Glu Ser Asp Gly Ser Gln Lys Val Asn Phe Leu Glu Tyr
65 70 75 80

Asn Ser Gly Tyr Gly Ile Ala Asp Thr Asn Thr Ile Gln Val Phe Val
85 90 95

Val Asp Pro Asp Thr Asn Asn Asp Phe Ile Ile Ala Gln Trp Asn
100 105 110

<210> 9

<211> 113
<212> PRT
<213> *Flammulina velutipes*

<220>
<221> PEPTIDE
<222> (1)..(113)
<223> FIP amino acid sequence

<400> 9

Ser Ala Thr Ser Leu Thr Phe Gln Leu Ala Tyr Leu Val Lys Lys Ile
1 5 10 15

Asp Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Thr Pro Ser Ser Tyr
20 25 30

Ile Asp Asn Leu Thr Phe Pro Lys Val Leu Thr Asp Lys Lys Tyr Ser
35 40 45

Tyr Arg Val Val Val Asn Gly Ser Asp Leu Gly Val Glu Ser Asn Phe
50 55 60

Ala Val Thr Pro Ser Gly Gly Gln Thr Ile Asn Phe Leu Gln Tyr Asn
65 70 75 80

Lys Gly Tyr Gly Val Ala Asp Thr Lys Thr Ile Gln Val Phe Val Val
85 90 95

Pro Asp Thr Gly Asn Ser Glu Glu Tyr Ile Ile Ala Glu Trp Lys Lys
100 105 110

Thr

<210> 10
<211> 49
<212> DNA
<213> Artificial

<220>
<223> primer

<220>
<221> primer
<222> (1)..(49)
<223> forward primer of FIP

```
<400> 10
aaaaactcga gaaaagagag gctgaagcta tgtccgacac tgccttgat 49
```

<210>	11
<211>	31
<212>	DNA
<213>	Artificial

```
<220>
<223> primer
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```
<220>
<221> primer
<222> (1)..(31)
<223> reverse primer of FIP
```

```
<400> 11
aaaaacacgt gtcaactagt tagttccatt g 31
```

<210>	12
<211>	315
<212>	DNA
<213>	Artificial

<220>
<223> recombinant DNA sequence for encoding recombinant protein

```
<220>
<221> CDS
<222> (22)..(315)
<223> recombinant protein containing alpha-factor and partial FIP
```

```

<400> 12
cggtacccg g gatccaaac g atg aga ttt cct tca att ttt act gca gtt      51
               Met Arg Phe Pro Ser Ile Phe Thr Ala Val
                   1               5               10

```

ttt	ttc	gca	gca	tcc	tcc	gca	tta	gct	gct	cca	gtc	aac	act	aca	aca	99
Leu	Phe	Ala	Ala	Ser	Ser	Ala	Leu	Ala	Ala	Pro	Val	Asn	Thr	Thr	Thr	
				15				20						25		

gaa gat gaa acg gca caa att ccg gct gaa gct gtc atc ggt tac tca 147
Glu Asp Glu Thr Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser
30 35 40

gat tta gaa ggg gat ttc gat gtt gct gtt ttg cca ttt tcc aac agc 195
Asp Leu Glu Gly Asp Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser
45 50 55

aca aat aac ggg tta ttg ttt ata aat act act att gcc agc att gct 243
Thr Asn Asn Gly Leu Leu Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala
60 65 70

gct aaa gaa gaa ggg gta tct ctc gag aaa aga gag gct gaa gct atg 291
 Ala Lys Glu Glu Gly Val Ser Leu Glu Lys Arg Glu Ala Glu Ala Met
 75 80 85 90

tcc gac act gcc ttg atc ttc agg 315
 Ser Asp Thr Ala Leu Ile Phe Arg
 95

<210> 13
 <211> 98
 <212> PRT
 <213> Artificial

<220>
 <223> Synthetic Construct

<400> 13

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
 1 5 10 15

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
 20 25 30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
 35 40 45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
 50 55 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
 65 70 75 80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Met Ser Asp Thr Ala Leu Ile
 85 90 95

Phe Arg

<210> 14
 <211> 624
 <212> DNA
 <213> Artificial

<220>
 <223> recombinant sequence

<220>

<221> CDS
 <222> (22)..(624)
 <223> recombinant sequence comprising alpha-factor and FIP

<400> 14

cggtacccgg ggatccaaac g atg aga ttt cct tca att ttt act gca gtt	51
Met Arg Phe Pro Ser Ile Phe Thr Ala Val	
1 5 10	
tta ttc gca gca tcc tcc gca tta gct gct cca gtc aac act aca aca	99
Leu Phe Ala Ala Ser Ser Ala Leu Ala Ala Pro Val Asn Thr Thr Thr	
15 20 25	
gaa gat gaa acg gca caa att ccg gct gaa gct gtc atc ggt tac tca	147
Glu Asp Glu Thr Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser	
30 35 40	
gat tta gaa ggg gat ttc gat gtt gct gtt ttg cca ttt tcc aac agc	195
Asp Leu Glu Gly Asp Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser	
45 50 55	
aca aat aac ggg tta ttg ttt ata aat act act att gcc agc att gct	243
Thr Asn Asn Gly Leu Leu Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala	
60 65 70	
gct aaa gaa gaa ggg gta tct ctc gag aaa aga gag gct gaa gct atg	291
Ala Lys Glu Glu Gly Val Ser Leu Glu Lys Arg Glu Ala Glu Ala Met	
75 80 85 90	
tct gat act gct ttg att ttc aga ttg gct tgg gat gtt aag aag ttg	339
Ser Asp Thr Ala Leu Ile Phe Arg Leu Ala Trp Asp Val Lys Lys Leu	
95 100 105	
tct ttc gat tac act cca aac tgg ggt aga ggt aac cca aac aac ttc	387
Ser Phe Asp Tyr Thr Pro Asn Trp Gly Arg Gly Asn Pro Asn Asn Phe	
110 115 120	
att gat act gtt act ttc cca aag gtt ttg act gat aag gct tac act	435
Ile Asp Thr Val Thr Phe Pro Lys Val Leu Thr Asp Lys Ala Tyr Thr	
125 130 135	
tac aga gtt gct gtt tct ggt aga aac ttg ggt gtt aag cca tct tac	483
Tyr Arg Val Ala Val Ser Gly Arg Asn Leu Gly Val Lys Pro Ser Tyr	
140 145 150	
gct gtt gaa tct gat ggt tct caa aag gtt aac ttc ttg gaa tac aac	531
Ala Val Glu Ser Asp Gly Ser Gln Lys Val Asn Phe Leu Glu Tyr Asn	
155 160 165 170	
tct ggt tac ggt att gct gat act aac act att caa gtt ttc gtt gtt	579
Ser Gly Tyr Gly Ile Ala Asp Thr Asn Thr Ile Gln Val Phe Val Val	
175 180 185	
gat cca gat act aac aac gat ttc att att gct caa tgg aac tga	624
Asp Pro Asp Thr Asn Asn Asp Phe Ile Ile Ala Gln Trp Asn	
190 195 200	

<210> 15
<211> 200
<212> PRT
<213> Artificial

<220>
<223> Synthetic Construct

<400> 15

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
1 5 10 15

Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
20 25 30

Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
35 40 45

Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
50 55 60

Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
65 70 75 80

Ser Leu Glu Lys Arg Glu Ala Glu Ala Met Ser Asp Thr Ala Leu Ile
85 90 95

Phe Arg Leu Ala Trp Asp Val Lys Lys Leu Ser Phe Asp Tyr Thr Pro
100 105 110

Asn Trp Gly Arg Gly Asn Pro Asn Asn Phe Ile Asp Thr Val Thr Phe
115 120 125

Pro Lys Val Leu Thr Asp Lys Ala Tyr Thr Tyr Arg Val Ala Val Ser
130 135 140

Gly Arg Asn Leu Gly Val Lys Pro Ser Tyr Ala Val Glu Ser Asp Gly
145 150 155 160

Ser Gln Lys Val Asn Phe Leu Glu Tyr Asn Ser Gly Tyr Gly Ile Ala
165 170 175

Asp Thr Asn Thr Ile Gln Val Phe Val Val Asp Pro Asp Thr Asn Asn
180 185 190

Asp Phe Ile Ile Ala Gln Trp Asn
195 200